Exhibit H

Tissue factor inhibition and clinical trial results of tissue factor pathway inhibitor in sepsis

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Tissue factor mediated pathways leading to microvascular thromboses and endothelial activation appear to play an important role in the development of multiple organ failure associated with severe sepsis. Tissue factor pathway inhibitor (TFPI) is an endogenous inhibitor of tissue factor associated coagulation cascades. In experimental models of severe sepsis, treatment with TFPI results in significant reduction in mortality. Similarly, a recently completed Phase II 210-patient study comparing placebo and infusions of TFPI showed trends toward a relative reduction in day 28 all-cause mortality in TFPI treated patients. These data suggest that coagulation cascades involving tissue factor contribute to organ dysfunction in critically ill septic patients. TFPI may be a useful therapy in improving outcome of severe sepsis. (Crit Care Med 2000; 28[Suppl.]:S31–S33)

Key Words: tissue factor; tissue factor pathway inhibitor; sepsis; coagulation; endothelium

Disseminated intravascular coagulation is common in critically ill septic patients and is associated with increased mortality (1, 2). Thrombin generation by tissue factor mediated coagulation pathways results in the formation of fibrin clots, endothelial activation to produce proinflammatory mediators, and microvascular thromboses (3). Such activation of clotting cascades, with associated endothelial involvement, appears to play a significant role in the development of multiple organ failure associated with severe sepsis (4, 5).

Tissue factor pathway inhibitor (TFPI) is an endogenous inhibitor of the tissue factor mediated, or extrinsic, pathway of blood coagulation (6, 7). TFPI forms an inhibitory quaternary complex with tissue factor, factor VIIa, and factor Xa, thereby blocking the generation of thrombin from prothrombin (Fig. 1). The ability of TFPI to inhibit the coagulation cascade at a very proximal point may provide advantages to this molecule, compared with other antithrombotic agents, in terms of inhibiting thrombin generation.

TFPI is a 276 amino acid glycoprotein (Fig. 2). It has an acidic aminoterminal region followed by three tandem domains that are homologous to Kunitz-type protease inhibitors. TFPI has been expressed in genetically engineered bacteria and mammalian cells. The recombinant protein, referred to as rTFPI, differs from the native protein by a single additional alanine residue at the amino terminus and when expressed in bacteria, the recombinant protein is not glycosylated.

Studies of rTFPI in Animal Models. Studies of treatment with rTFPI in animal models of severe sepsis, such as *Escherichia coli*-infused baboons (8) or rabbits with intraperitoneal infection (9), demonstrate significant reduction in mortality. These studies showed that administration of rTFPI after the infectious insult also could improve outcome. In addition, rTFPI improved abnormal hematologic variables, consistent with disseminated intravascular coagulation, and also reduced circulating levels of cytokines, such as interleukin-6. Interestingly, even extremely low doses of rTFPI (0.05 μg/kg/min), without apparent anticoagulant effects, were effective in rabbit intraperitoneal infection models (9). The mechanism for this beneficial effect of extremely low dose TFPI remains undetermined.

Studies of TFPI and rTFPI in Healthy Humans. Studies of TFPI and rTFPI were conducted to elucidate the pharmacokinetic/pharmacodynamic properties of TFPI in healthy humans. The first study (10) determined the effects of two different doses of TFPI on endotoxin-induced coagulant, fibrinolytic, and cytokine responses in healthy humans. The second study (11) validated the importance of hepatic clearance in the pharmacokinetic/pharmacodynamic profile of TFPI. Both studies clearly demonstrated the dose-related anticoagulant effect of TFPI.

In the endotoxin challenge study (10), 16 healthy men were studied in a double-blind, randomized, placebo-controlled crossover study. Each subject was studied on two different occasions. The 16 subjects received a bolus intravenous injection of 4 mg/kg endotoxin followed by a 6-hr continuous infusion of TFPI or placebo. Eight subjects received a bolus of 0.0125 mg/kg TFPI followed by 0.05 mg/kg/hr (low-dose group), and the other eight subjects received a bolus of 0.05 mg/kg TFPI followed by 0.2 mg/kg/hr (high-dose group).

As with earlier endotoxin challenge studies (12, 13), a bolus of endotoxin induced activation of coagulation, activation and subsequent inhibition of fibrinolysis, and release of proinflammatory and anti-inflammatory cytokines. rTFPI infusion induced a dose-dependent attenuation of thrombin generation as assessed by plasma thrombin-antithrombin and F_{1+2} complexes. Endotoxin-induced changes in the fibrinolytic system and cytokine levels were not altered by either low or high-dose rTFPI. There was a

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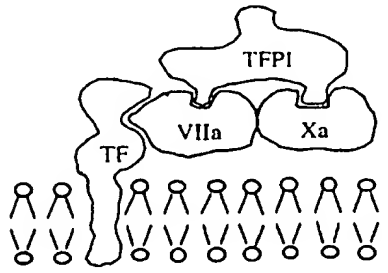
- Natural anticoagulan
- Inhibits Tissue Factor Pathway by forming a quanternary complex



- TF is exposed / upregulated (adventitia, monocytes, endothelium)
- TF converts VII to VIIa
- VIIa converts X to Xa
- TFPI preforms a complex with Xa
- TFPI/Xa then binds TF-VIIa

Figure 1. Tissue factor pathway inhibitor biology. *TFPI*, tissue factor pathway inhibitor; *TF*, tissue factor.

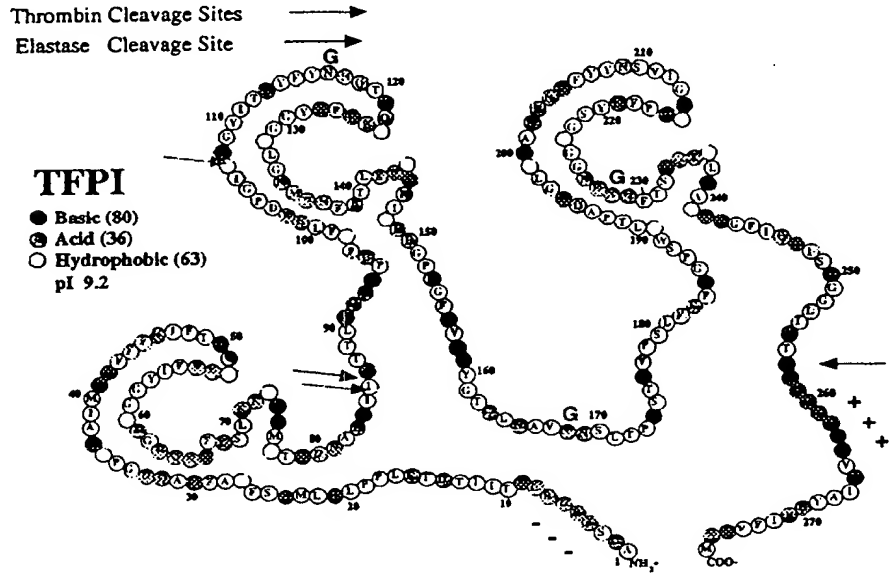


Figure 2. Structure and amino acid sequence of tissue factor pathway inhibitor.

slight, but not statistically significant, reduction in interleukin-6 levels by rTFPI.

The data are consistent with the observation that low-grade endotoxemia induces the activation of cell populations such as monocytes, macrophages, and neutrophils to produce cytokines. Higher doses of endotoxin result in endothelial activation as well. In the baboon bacteremia model, the rTFPI-associated decrease in cytokine production is presumed to reflect effects on endothelial cells. Such attenuation of proinflammatory cytokine generation is thought to indicate that rTFPI enhances resolution of microvascular injury, decreases endothelial cell procoagulant activity/thrombosis, and, via such mechanisms, prevents organ failure and ultimately death.

Preclinical experiments indicated that TFPI is cleared hepatically (14). However, confirmation of TFPI hepatic clearance in humans is less well established. One strategy to confirm the importance of liver blood flow in the pharmacokinetic/pharmacodynamic profile of TFPI used the documented effect of exercise-induced reduction in liver blood flow on TFPI circulating levels and possibly prolongation of prothrombin time and activated partial thromboplastin time. The study design included a two-way open-label, randomized, crossover study in healthy male volunteers (11). The subjects in the two treatment groups received a continuous intravenous infusion of TFPI (0.2 mg/kg/hr) concurrently with intravenous sorbitol (50 mg/min) for 4 hrs. Sorbitol was used as a biomarker for liver blood flow. The subjects were randomized to remain supine or to exercise on a bicycle ergometer for the first 30 mins of the third hour of the infusion.

In these experiments, the reduction in liver blood flow by exercise increased circulating TFPI levels and induced only a slight and variable prothrombin time and activated partial thromboplastin time increases. These observations are consistent with the hepatic clearance of TFPI and suggest that prothrombin time is an acceptable indirect marker of TFPI activity.

Tissue factor pathway inhibitor may be a useful therapy in improving outcome of severe sepsis.

Studies of TFPI in Septic Patients. Several small phase I and II studies have examined the use of rTFPI in normal humans and patients with sepsis. In the initial study, which enrolled 14 patients, 5 received placebo, 5 were given rTFPI at 0.33 mg/kg/hr, and 4 were given rTFPI at 0.66 mg/kg/hr. Three subjects (one in the rTFPI 0.33 mg/kg/hr group and two in the 0.66 mg/kg/hr group) had greater than expected anticoagulation (prolonged PT) while receiving active infusion, and there was an apparent increase in serious adverse events involving bleeding in the rTFPI groups.

A subsequent study evaluated lower doses of rTFPI. In this study, rTFPI doses of 0.025–0.1 mg/kg/hr were used in conjunction with bedside monitoring of prothrombin time. Compared to the placebo group, there was no evidence of an increased rate of serious adverse events or bleeding events, indicating that such doses of rTFPI could be safely administered to patients with severe sepsis.

A recently completed phase II study compared placebo and 0.025 mg/kg/hr or 0.05 mg/kg/hr rTFPI in 210 patients with severe sepsis. The treatment and placebo arms did not differ appreciably with respect to all adverse events or those related to bleeding. There was a trend toward reduction in all causes of mortality in the rTFPI-treated group. These results

suggest that rTFPI is of benefit in severely ill, septic patients and have led to the initiation of a large international phase III study of rTFPI for this indication.

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Question and Answer Session After Scientific Review

Jean-Louis Vincent. The tissue factor pathway is an important trigger of coagulation activation, but once the system is activated it doesn't play a role anymore. So if we still want to intervene once coagulation alterations have developed, wouldn't it be better to intervene with antithrombin III or activated protein C?

Edward Abraham. You are asking exactly the right question, but we really do not have an answer yet. Tissue factor is at the bottom of the whole cascade and may be very important if it is continually activated in sepsis. However, the relative importance of this is unknown. An important issue is that patients are exquisitely sensitive to tissue factor plasminogen inhibitor (TFPI), so if TFPI works only by inhibiting the coagulation cascade, it will be dose-limited. The real question is "Why does TFPI work in rabbits at low doses, and could a similar mechanism of action apply to humans also?"

Charles Esmon. TFPI is one of the most complicated inhibitors because it exists in two forms–a soluble form, which is what we measure in plasma, and a form bound to endothelial cells. We have no idea what the relative distribution of TFPI is in the microvasculature of patients or rabbits. When we give TFPI exogenously, we do not know where it is supposed to work. Another issue to consider is that the coagulation system is heavily inhibited. If you take away the initiator in the system, Xa and thrombin will have a half-life of only a few seconds under normal circumstances, so the system will shut itself down. The distribution of TFPI may have a lot to do with the outcome that we have seen in rabbits and humans. The ability of TFPI to bind to the endothelium in the time frame that we are considering could also be very important.

C. Erik Hack. I think there is a basic mistake in the design of this trial, which is that patients with certain prolongations of clotting times were excluded. Animal data for TFPI, particularly in the baboon studies, was obtained from disseminated intravascular coagulation (DIC)-driven models, but similar patients were excluded from the TFPI study.

Edward Abraham. I have exactly the same concern, to be perfectly honest. One needs to identify the probable mechanism of action that is being tested with TFPI. If you say that it is an anti-DIC drug, then of course you want to choose patients with elevations in international normalized ratio and prothrombin time and clinical evidence of DIC to begin with. If, however, you say that it has some other mechanism of action, then you need to define what that mechanism of action is and pick patients who have that abnormality. I think that is exactly where the conflict is at the present time.